Procedures for the Semi-Automated Extraction of DNA

1 Scope

These procedures apply to DNA personnel performing semi-automated extraction and purification of deoxyribonucleic acid (DNA) using the QIAcube®, EZ1® Advanced XL (EZ1®), and/or QIAsymphony® SP in the DNA Casework Unit (DCU) or Biometrics Analysis Unit (BAU) and DNA personnel that perform the associated quality control procedures.

2 Equipment/Materials/Reagents

Materials

- General laboratory supplies (e.g., tubes, pipettes)
- Costar® spin baskets, or equivalent (*for differential extractions*)
- Qiagen® Lyse & Spin Baskets and Collection Tubes, or equivalent (*for normal extractions*)
- QIAcube®
- QIAcube® consumables (e.g., 1000 µL wide-bore filter-tips, reagent bottles, rotor adapters, QIAcube®-compatible 1.5 mL microcentrifuge tubes)
- EZ1® Advanced XL
- EZ1® DNA Investigator Kit (e.g., filter tips, tip holders, elution tubes, reagent cartridges)
- QIAsymphony® SP
- QIAsymphony® DNA Investigator Kit (e.g., reagent cartridges, piercing lid, enzyme rack, Reuse Seal Strips)
- QIAsymphony® SP consumables (8-Rod Covers, Sample Prep Cartridges, 200 µL tips, and 1500 µL tips)
- Speed-Vac, Vacufuge Concentrators, or equivalent

Reagents

- Buffer ATL
- Buffer G2 *(for EZ1 normal lysis and EZ1 male fraction only)*
- 1M Dithiothreitol (DTT)
- Proteinase K
- Reagent Grade Water

3 Standards and Controls

At least one extraction control (i.e., reagent blank) must be processed in parallel with each extraction batch.

For evaluation of the extraction controls, refer to the appropriate interpretation procedure of the *DNA Procedures Manual*.

4 Procedures

Refer to the DNA Procedures Introduction (DNA QA 600) for applicable general precautions and cleaning instructions.

Ensure the appropriate fields (i.e., instruments, reagents) in the Sample Tracking and Control Software (STACS) are completed from any network computer, as necessary.

4.1 Normal Lysis

4.1.1	Create master mix using the appropriate recipe listed below.	

If precipitate has formed in the Buffer ATL, heat it, generally at 56°C, until precipitate is no longer visible.

Normal Lysis Master Mix

EZ1® Advanced XL

Reagent	µL per sample
Buffer G2	470
Pro K	15
DTT	15

QIAsymphony®

Reagent	µL per sample
Buffer ATL	460
Pro K	20
DTT	20

The samples should be in Lyse & Spin baskets in corresponding tubes.

4.1.2	Add 450 µL master mix.	

4.1.3	Incubate the tubes in an incubator at 56°C with agitation (generally 200 rpm) for ~1 hour.	

4.1.4	Spin the tubes (generally between 9,000 and 13,000 rpm for 5 minutes). Discard the basket.	

If the lysate does not completely flow through the basket, additional spins may be added. If necessary, lysate remaining in the basket may be manually transferred to the sample tube. Additional manipulations will be recorded in the case notes. If the volume in the sample tube is significantly different than the expected volume a case note will be made.

4.1.5	Process the lysates on the EZ1® following the steps in section 4.3 or QIAsymphony® SP following the steps in section 4.4, as appropriate.	

4.2 Differential Lysis

4.2.1	Create the epithelial (F) fraction master mix.	

If precipitate has formed in the Buffer ATL, heat it, generally at 56°C, until precipitate is no longer visible.

Female Fraction Lysis Master Mix

Reagent	µL per sample
Buffer ATL	160
Reagent Grade Water	320
Pro K	20

The samples should be in QIAcube® compatible tubes. Lyse & Spin baskets must NOT be used for differential extractions.

4.2.2	Add 450 µL master mix.	

4.2.3	Incubate the tubes at 56°C with agitation (generally 900 rpm) for ~1.5 hours.	

Generally, differential incubations are done in a thermomixer.

4.2.4	If necessary, quick spin and transfer the substrate to a basket. Spin the tubes (generally between 9,000 and 13,000 rpm for 5 minutes). Discard the basket.	

4.2.5	Ensure consumables, reagent grade water, and lysate tubes are properly loaded onto the QIAcube® instrument.	

Consumables include: bottles containing reagent grade water, 1000 µL wide-bore tips, rotor adapters, and F fraction collection tubes. A shaker rack plug should also be in place next to each F fraction collection tube.

Appendix A has additional guidance for loading the QIAcube®.

4.2.6	Initiate the "Separate and Lyse 12A Mod" protocol on the QIAcube®.	

The bottle in position 1 will contain reagent grade water, not Buffer G2 as prompted.
Be aware that lysates are 450 µL, not 500 µL as referenced in the QIAcube® prompt.

4.2.7	At the completion of the run, tubes containing the F fraction should be removed and capped. Refill consumables and reagent grade water as necessary.	

The sperm (M) fractions continue processing on the QIAcube®.

Processing of the F fraction resumes at section 4.3, either independently or with the M fraction.

4.2.8	Initiate the "Separate and Lyse 12B Mod" protocol on the QIAcube®.	

The bottles will contain reagent grade water, not Buffer G2 as prompted.

4.2.9	At the completion of the run, remove and cap the tubes containing the M fraction(s).	
4.2.10	A slide for microscopy may be prepared from the M fraction according to Serology Procedure 112 (Sero 112).	
4.2.11	Ensure the M fraction master mix is created.	

Male Fraction Lysis Master Mix

EZ1® Advanced XL

Reagent	µL per sample
Buffer G2	375
Pro K	24
DTT	94

QIAsymphony®

Reagent	µL per sample
Buffer ATL	375
Pro K	24
DTT	94

4.2.12	Add 450 µL master mix to each M fraction tube.	
4.2.13	Vortex, quick spin, and incubate the tubes at 70°C with agitation (generally 900 rpm) for ~10 minutes.	
4.2.14	Vortex the tubes vigorously (~10 seconds) and quick spin.	
4.2.15	Process the lysates on the EZ1® following the steps in section 4.3 or QIAsymphony® SP following the steps in section 4.4, as appropriate.	

Ensure waste is disposed of properly.

The water bottles on the QIAcube® should be emptied at the end of each day of use.

4.3 Processing Lysates on the EZ1® Advanced XL

Lysates may be stored refrigerated for up to 6 days prior to processing on the EZ1®. Ensure that no precipitate remains in the lysates prior to processing.

4.3.1	Ensure the EZ1® is UV irradiated for 20 minutes prior to initial use each day.	

If precipitate has formed in the EZ1® cartridges, heat them, generally at 56°C, until precipitate is no longer visible.

If necessary, transfer the lysate to an EZ1® compatible tube.

4.3.2	If appropriate, two lysates may be combined prior to loading on the EZ1®.	

4.3.3	Ensure consumables, lysate tubes, and barcoded elution tubes are properly loaded onto the EZ1®. See Figure 1.	

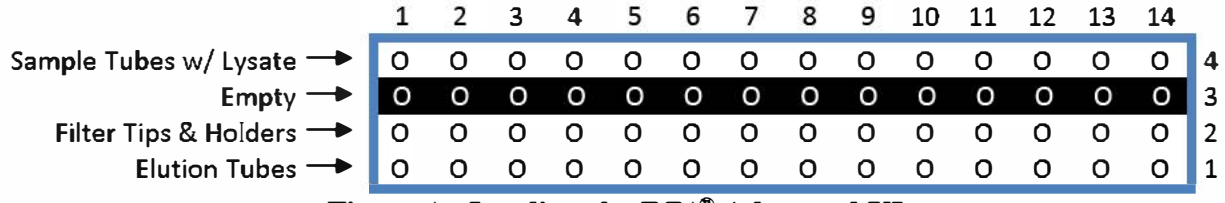


Figure 1 - Loading the EZ1® Advanced XL

4.3.4	Ensure the Large Volume protocol with elution into 50 µL of water has been selected and start the EZ1®.	

Disregard the reference to MTL Buffer in the prompts on the EZ1®.

4.3.5	At the completion of the run, remove and cap elution tubes.	

If the volume in the elution tube is significantly different than the expected volume a case note will be made.

4.3.6	If necessary, combine appropriate extracts.	

If combined samples will be concentrated using the Speed-Vac, a maximum of eight 50 µL extracts may be combined into one tube.

4.3.7	Ensure waste is disposed of properly. Ensure the piercing units on the EZ1® are cleaned after daily use and clean the tray, worktable, and racks, if needed.	

Bleach should not be used on the QIAcube® or the EZ1® Advanced XL. *70% ethanol should be used to clean instrument surfaces.*

4.4 Processing Lysates on the QIAsymphony® SP

Lysates may be stored refrigerated for up to 6 days prior to processing on the QIAsymphony® SP. Ensure that no precipitate remains in the lysates prior to processing.

4.4.1	Remove the waste chute and tip park station. Ensure the QIAsymphony® SP deck is empty prior to UV irradiation and that the instrument is UV irradiated for at least 15 minutes prior to initial use each day.	

4.4.2	Ensure the QIAsymphony® SP is prepared for the run. • Load consumables, waste chute, tip park station, sample tubes containing lysate, and barcoded elution tubes. • Select the Custom Protocol. • If necessary, change the tube type.* • Select the appropriate elution volume, generally 100 µL. • Associate the appropriate sample racks with the corresponding elution tube racks.	

** If the tube volume default is 2 mL, the setting for any position in the sample rack that contains a 1.5 mL tube (e.g., QIAcube® tube) should be changed to reflect the appropriate tube volume.*

Appendix B has additional guidance for loading the QIAsymphony®SP.

4.4.3	Start the QIAsymphony® SP.	

4.4.4	At the completion of the run, remove and cap elution tubes. Verify the volume in the elution tubes prior to discarding the components on the deck.	

Empty sample tubes may be discarded after processing of the sample rack. If significant volume remains in a sample tube, it should be retained until elution volume is verified.

If eluate is not present in an elution tube, the QIAsymphony®SP User Manuals may be referenced for troubleshooting and potential sample recovery procedures.

4.4.5	If necessary, combine appropriate extracts.	

If combined samples will be concentrated using the Speed-Vac, a maximum of four 100 µL extracts may be combined into one tube.

Ensure waste is properly disposed (i.e., lysis tubes, tip waste, and liquid waste).

All plastics and reagents should be removed following daily use. The reagent cartridge should be capped prior to storage or disposal.

The deck, back tray, magnetic head guards, tip guards, tip park station, and waste chute should be cleaned following daily use with 70% isopropanol (use water, not alcohol, on the hood of the instrument).

Bleach should not be used on the QIAsymphony®SP.

Refer to the QIAcube® User Manual and the QIAsymphony®SP User Manuals for guidance on instrument maintenance and deeper cleaning, as required.

4.5 Concentrating Extracted Samples Using the Speed-Vac or Vacufuge

The Speed-Vac flask should be empty and dry, and the flask seal should be tight.

The Speed-Vac should be turned on ~45 minutes prior to use.

Ensure the gasket on the centrifuge is in its proper position and that the rotor is properly tightened during sample processing.

4.5.1	Samples from questioned items (and corresponding reagent blanks) are typically concentrated using a Speed-Vac or Vacufuge and reconstituted with 15 µL with reagent grade water.	

If an alternate volume of reagent grade water is used to reconstitute, it will be documented.

Samples from known items (and corresponding reagent blanks) typically do not require concentration.

On the Speed-Vac with the heat set to "High", a 50 µL extract may take ~30-40 minutes to dry and a 100 µL extract may take ~60 minutes to dry. Samples should not be dried on "High" for more than four hours (maximum starting volume of ~400 µL).

On the Vacufuge with a setting of 60ºC, a 50 µL extract takes about 45 minutes.

Alternatively, combining/concentrating can be done using a Microcon Filter rather than a Speed-Vac.

4.6 Combining/Concentrating Extracted Samples Using a Microcon Filter

4.6.1	Vortex and quick spin the extract tubes. Transfer the extract for each sample being combined/concentrated into a labeled microcon assembly.	

Corresponding reagent blanks must also be microconned.

4.6.2	Spin the tubes to draw the fluid through the membrane (generally between 6,000 and 8,000 rpm for 10 minutes).	

Speed and time may be increased to draw fluid through membrane, but to avoid damage to the membrane, excess speed and time should not be used.

If additional spins do not reduce the volume, the affected sample(s) may continue with processing at step 4.6.4. Record the final volume.

4.6.3	Add reagent grade water (generally 15 µL).	
4.6.4	Invert microcon into a new, labeled tube.	
4.6.5	Spin the tubes (generally between 9,000 and 13,000 rpm for 5 minutes).	
4.6.6	Ensure the final tubes are barcoded.	

5 Sampling or Sample Selection

Not applicable.

6 Calculations

Not applicable.

7 Measurement Uncertainty

Not applicable.

8 Limitations

The quantity and quality of the DNA present within any biological material ultimately determines if a DNA extraction is successful.

9 Safety

9.1 All evidence containing or contaminated with blood or other potentially infectious materials will be considered infectious regardless of the perceived status of the source individual or the age of the material. Follow the "Safe Work Practices and Procedures," "Bloodborne Pathogen (BBP) Exposure Control Plan (ECP)," "Personal Protective Equipment Policy," and "Chemical Hygiene Plan" sections of the *FBI Laboratory Safety Manual*.

9.2 Refer to the "Hazardous Waste Disposal" section of the *FBI Laboratory Safety Manual* for important information concerning proper disposal of the chemicals used in these procedures as well as the biohazardous wastes generated.

9.3 Procedural Specific Chemical Hazards:

- Solutions of Proteinase K can be irritating to mucous membranes. Use eye protection when handling.

- EZ1® reagent cartridges, QIAsymphony® reagent cartridges, and QIAsymphony® liquid waste contain ethyl alcohol and guanidine salts which are hazardous materials. Solutions containing guanidine salts will generate toxic fumes when combined with bleach. Use approporiate care and wear appropriate protective clothing and eyewear when handling. Be careful not to expose face or hands to splashes. Dispose of EZ1® and QIAsymphony® consumables in appropriate waste containers.

10 References

FBI Laboratory Safety Manual

DNA Procedures Manual

Qiagen®. *QIAcube® User Manual*, June 2008.

Qiagen®. *EZ1® Advanced XL User Manual*. May 2009.

Qiagen®. *EZ1® DNA Investigator Handbook.* April 2009.

Qiagen®. *QIAsymphony® DNA Investigator Handbook.* February 2013.

Qiagen®. *QIAsymphony®SP/AS User Manual – General Description*. May 2013.

Qiagen®. *QIAsymphony®SP/AS User Manual – Operating the QIAsymphony® SP*. April 2012.

Qiagen®. *QIAsymphony® SP/AS Management Console User Manual (software version 4.0)*. April 2012.

Qiagen®. *QIAsymphony® SP/AS Consolidated Operating Guide*. May 2013.

Rev. #	Issue Date	History
2	02/28/18	Revised scope. Added BAU. Updated references throughout. Added general introduction information to procedures. 4.3.7: Added EZ1 cleaning guidance in lieu of referring to user manual. Added Appendix C with instrument QC procedures from STACS instructions.
3	02/15/19	2 & 4.5: Added Vacufuge contentrator in addition to Speed vac and default settings. 4.1.4 & 4.3.5: Added allowance for additional spins and instruction to note any volume discrepancies after spins or elution. Appendix C: Changed QC samples to swabs.

Approval

Redacted - Signatures on File

DNA Technical Leader Date: 02/14/2019

Acting BAU Chief Date: 02/14/2019

DCU Chief Date: 02/14/2019

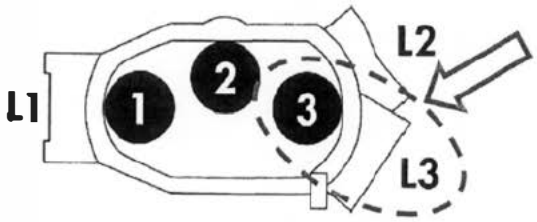
DSU Chief Date: 02/14/2019

QA Approval

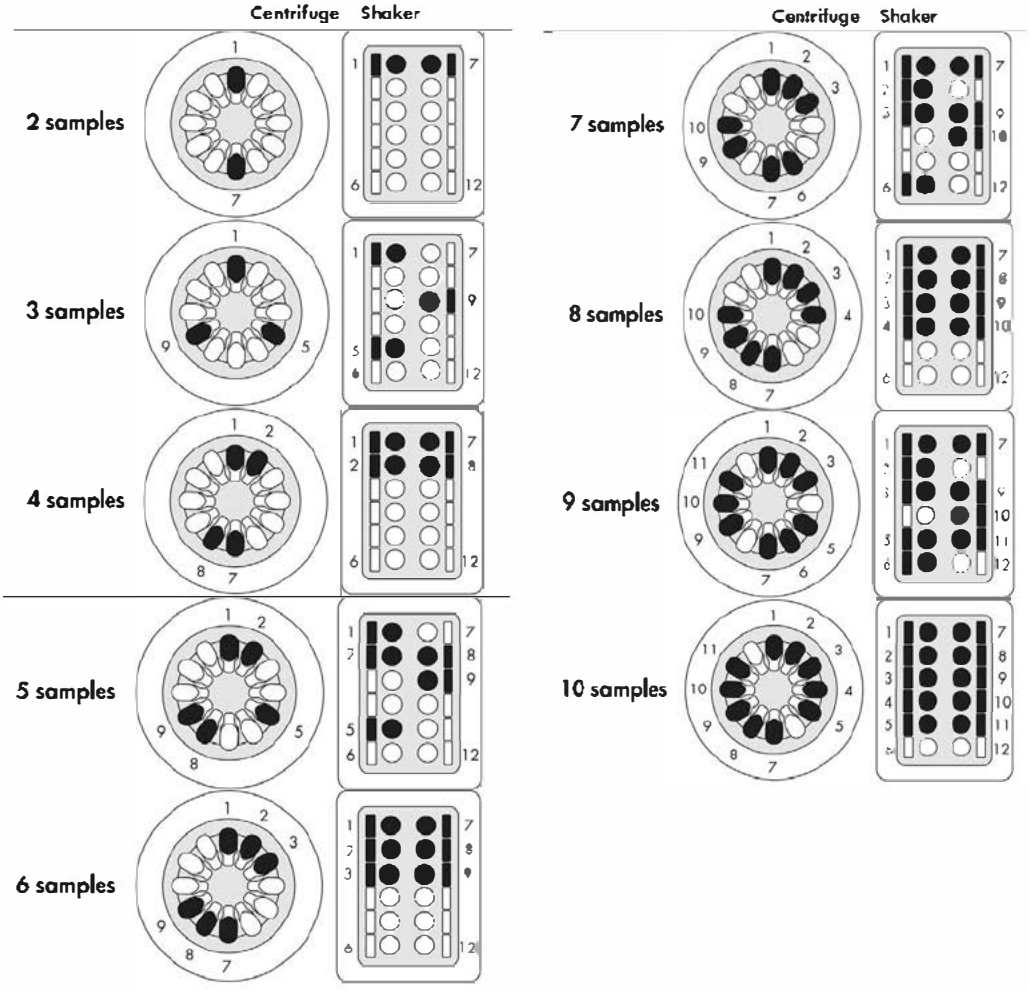
Quality Manager Date: 02/14/2019

Appendix A: *Loading the QIAcube®*

The lysate tubes fit into position 3 of the rotor adaptors, with their caps folded back and inserted into position L3.

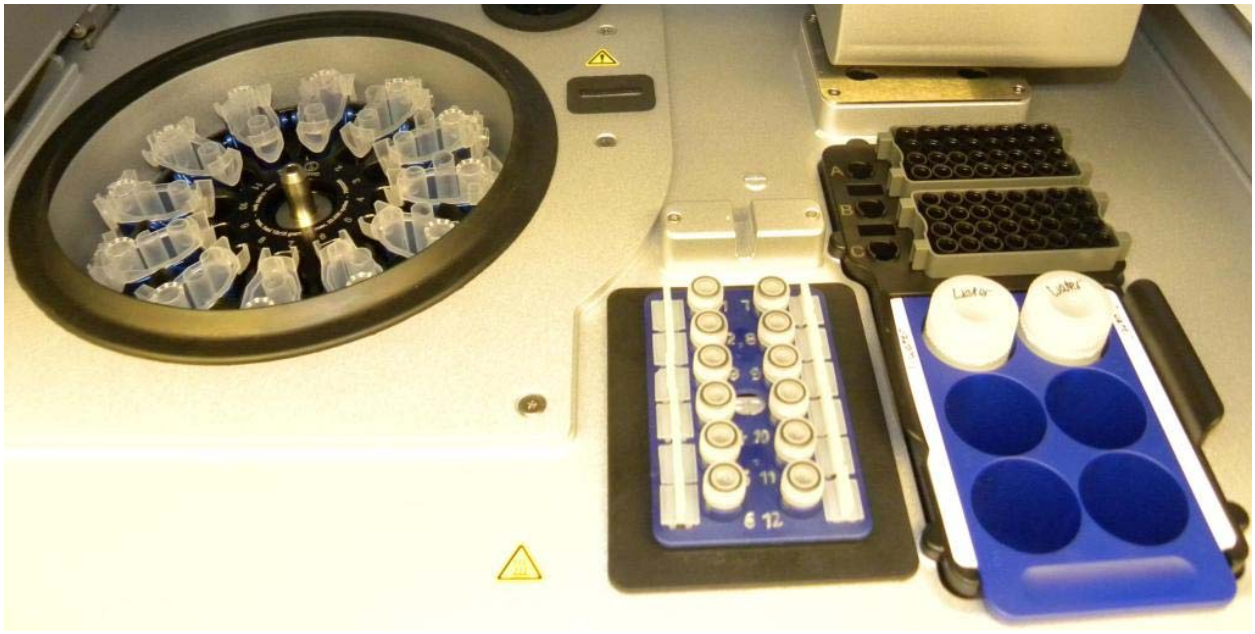


Rotor adaptors with lysate tubes must be distributed on the centrifuge for balance.



Appendix A: *Loading the QIAcube®(continued)*

An overview of the loaded QIAcube®.



Appendix B: *Loading the QIAsymphony®SP*

The QIAsymphony®*SP* is comprised of four drawers that can be loaded in any order.

A wizard is available from the main screen to assist in loading.

A. Sample drawer

Load barcoded tubes containing lysate into a sample rack(s), and then slide the sample rack(s) into the appropriate position(s) in the sample drawer.

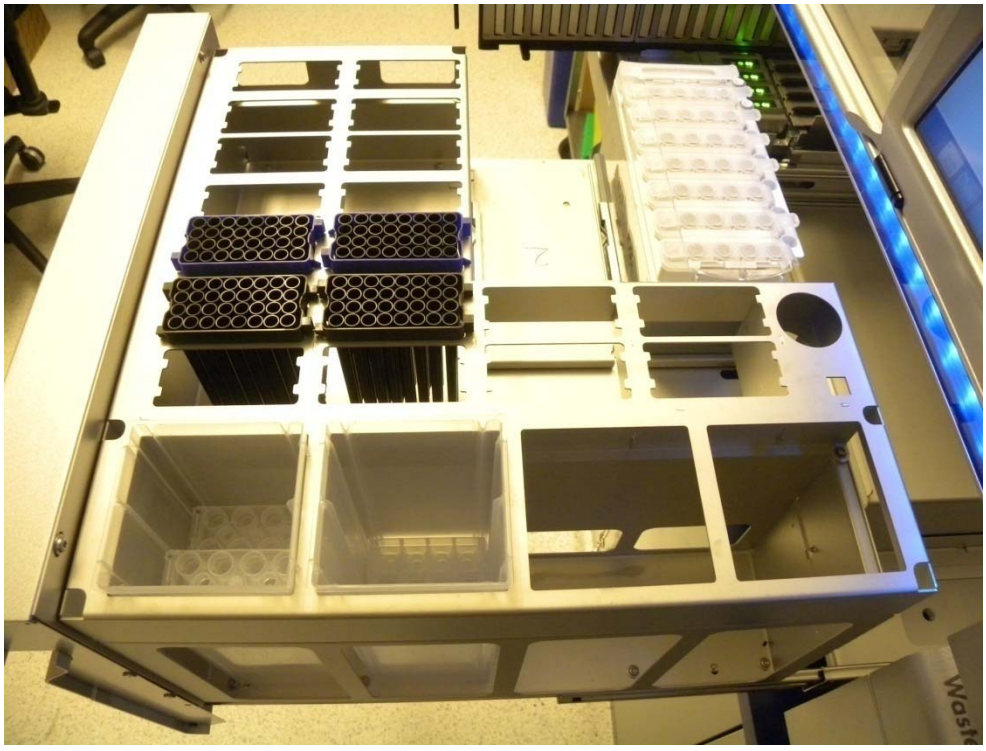
Appendix B:*Loading the QIAsymphony®SP (continued)*

B. Reagents and Consumables drawer

Load sufficient reagents and consumables to process the appropriate number of samples. This drawer will contain reagent cartridge(s), 200 µL tips, 1500 µL tips, sample prep cartridge(s), and 8-rod covers.

Reagent Cartridge preparation (in any order):

- Attach enzyme rack to the reagent cartridge.
- Add ~1600 µL of reagent grade water to both tubes in the enzyme rack.
- Place the reagent cartridge in the reagent cartridge holder.
- Remove the magnetic bead trough and vortex or shake for at least one minute. Replace the trough in the proper orientation. If first use, remove the foil cover. For subsequent uses, remove the Reuse SealStrip.
- If first use, place the piercing lid on top of the reagent cartridge. For subsequent uses, remove the reagent cartridge Reuse SealStrips prior to loading.

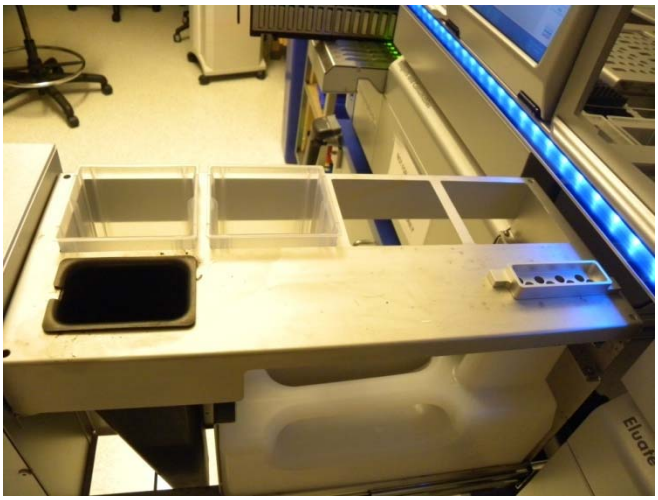


Appendix B: *Loading the QIAsymphony®SP (continued)*

C. Waste drawer

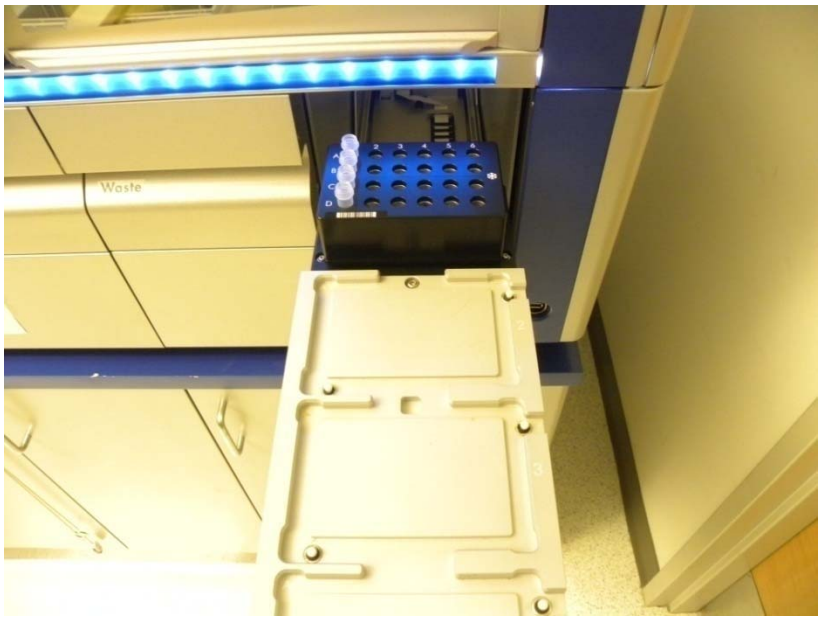
Ensure plastic vessels (empty 8-rod cover or sample prep cartridge containers), the waste chute, the liquid waste jug, and the tip park station are in place.

Ensure that the tip waste container in bottom right cabinet is not completely full.



D. Eluate drawer

Barcoded elution tubes should be loaded into an elution rack. The number and order of tubes loaded should correspond to the number and order of tubes containing lysate loaded in the sample drawer. The elution rack(s) are loaded back-to-front in column format.



Appendix C: Instrument General Maintenance and Performance Verification Procedures

Refer to the DNA procedure for instrument calibration and maintenance (i.e., DNA QA 608) for minimum frequency of performance verifications and additional requirements.

1. QIAcube® Robotic System (Extraction)

A. General Maintenance

There is no general maintenance required for the QIAcube®.

B. Performance Verification

A portion of a swab containing 5 µL female blood and 1 µL of a 10x dilution of semen (semen diluted with reagent grade water) with known typing results will be processed using the QIAcube® differential lysis procedure followed by extraction using the appropriate DNA standard operating procedure (SOP).

The mixture must yield a distinguishable M fraction containing predominantly male DNA with minimal carryover from the F fraction as determined by quantification and amplification. The mixture is expected to yield a distinguishable F fraction containing predominantly female DNA but may contain a mixture of male and female DNA depending on the sample.

If the sample does not produce a distinguishable M fraction the process should be repeated with a new sample. If a predominately male DNA containing M fraction is not accomplished after 2 attempts the Technical Leader (TL) will be consulted.

2. EZ1® Advanced XL Robotic System (Extraction)

A. General Maintenance

Quarterly: Inspect the O-rings and grease as needed.
Test Heating Block at 70°C.

B. Performance Verification

A swab containing 5 µL blood from a known donor will be processed using the Normal lysis procedure followed by the EZ1® extraction procedure found in the appropriate DNA SOP for each of the 14 channels of the EZ1 robot. Alternately, individual channels may be tested to verify performance as needed.

A sample from each channel must yield a concentration, determined by quantification, greater than the minimum value established for the specific donor batch on the EZ1® using a 50 µL elution volume. A channel, or channels, may be repeated if necessary.

If a channel, or channels, does not yield the appropriate concentration after 2 attempts the TL will be consulted.

3. QIAsymphony® SP Robotic System (Extraction)

A. General Maintenance

Monthly or at 1000 runs: Change O-rings

B. Performance Verification

A swab containing 5 µL blood from a known donor will be processed using the Normal lysis procedure followed by the QIAsymphony® extraction procedure found in the appropriate DNA SOP for each of the 4 channels of the QIAsymphony® instrument. Alternately, individual channels may be tested to verify performance as needed.

A sample from each channel must yield a concentration, determined by quantification, greater than the minimum value established for the specific donor batch on the QIAsymphony® using a 100 µL elution volume without TopElute fluid (TOPE). A channel, or channels, may be repeated if necessary.

If a channel, or channels, does not yield the appropriate concentration after 2 attempts the TL will be consulted.

4. Establishing the minimum value for Extraction Robot PV

Use an in service instrument and the appropriate extraction procedure to extract several samples from a blood card (i.e., FTA card) or swabs spotted with donor blood. The minimum quantitation value will be established by calculating the average quantitation of the extracted samples minus three standard deviations.